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The DCCT/EDIC Study: Epigenetic Clues After Three Decades

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COMMENTARY

The advent of insulin therapy in the 1920s prolonged life expectancy in patients with type 1 diabetes (T1D). However, along with extended lifespan came increased morbidity and mortality associated with the micro- and macrovascular complications of diabetes. Because early insulin treatment regimens did not anticipate the need for multiple daily injections for tight glycemic control, hyperglycemia was proposed as the culprit for these vascular complications.

In the 1980s, this hypothesis was tested in the Diabetes Control and Complications Trial (DCCT). The DCCT was a randomized trial of a cohort of recently diagnosed T1D patients that compared conventional insulin therapy at the time, which consisted of one or two daily injections, to an intensive multiple daily injection regimen along with strict monitoring of glycemia (1). In DCCT, patients in the intensive control arm achieved and maintained HbA_{1c} levels of approximately 7%, two percentage points lower than those in the conventional therapy group. At study end, improved glycemic control was associated with a lower incidence and progression of retinopathy, nephropathy, and neuropathy, thus providing evidence of a link between glycemia (defined by HbA_{1c} levels) and the incidence of diabetic microvascular complications (2).

The clinical benefits of intensive control were so clear that DCCT was terminated early and intensive insulin therapy was proposed as the new standard of care (3). The DCCT's conventional therapy group was switched to the more beneficial intensive regimen, and DCCT cohort began an observational follow-up phase—the Epidemiology of Diabetic Interventions and Complications (EDIC) study—which began in 1993 (4). EDIC aimed to gather information on the longer-term development of microvascular complications and more advanced diabetes morbidities that were not observed in DCCT due to its relatively short duration. During EDIC, the two former DCCT arms (intensive insulin therapy and conventional treatment) quickly converged with equalized HbA_{1c} levels (4). Despite the loss of separation in HbA_{1c} that was observed as early as the first year of EDIC, DCCT/EDIC, now entering its fourth decade, still shows persistent beneficial effects of earlier allocation to the DCCT's intensive therapy arm, as demonstrated by reductions in severe diabetes complications (5). The persistence of these benefits in the EDIC cohort implies "memory" of the DCCT's intensive versus conventional treatment regimen. Among the proposed mechanisms mediating this "glycemic" or "metabolic memory" is the occurrence of persistent epigenetic modifications induced by transitory hyperglycemia and subsequent oxidative stress (6) (reviewed in 7–9).

Epigenetic modifications involve transcriptionally suppressive cytosine DNA methylation (10) and histone post-translational modifications (PTMs). Histone PTMs can be transcriptionally permissive, such as histone H3 acetylation and lysine-4 methylation, or transcriptionally repressive, such as H3 lysine-9 methylation (11). DNA methylation and histone PTMs have been investigated as epigenetic modifications that are potentially associated with glycemic memory in experimental models ranging from primary endothelial cells (12,13) to zebrafish (14). Prolonged responses to transient exposure to high glucose also resulted in sustained expression of protein markers of high-glucose stress, including the NADPH oxidase subunit $p47^{phox}$ and fibronectin (15), indicating that epigenetic changes modulate both gene transcription and associated protein expression. An early, clinically oriented study comparing histone PTMs in lymphocytes from patients with T1D and control subjects provided evidence for an association between T1D and altered histone methylation on genes implicated in the pathophysiology of T1D (16). These findings suggested an association between epigenetic modifications and metabolic memory. However, whether epigenetic events play a clinically relevant role in

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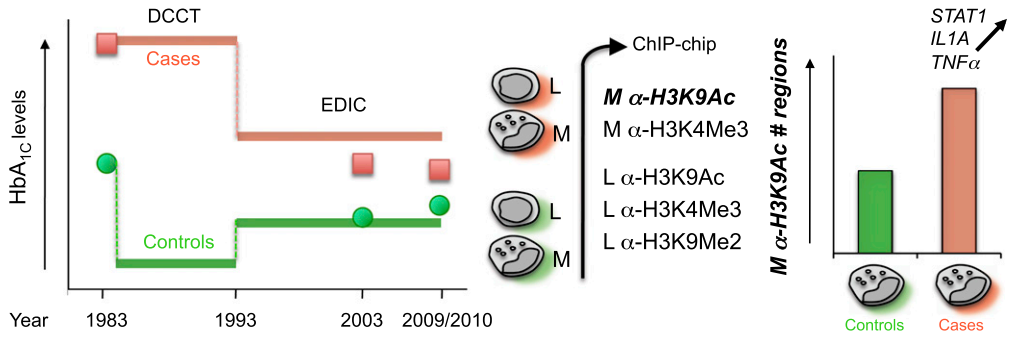

Figure 1—H3K9Ac is an epigenetic mark retained in monocytes from T1D patients who had conventional insulin therapy during DCCT. The graph on the *left* illustrates the HbA_{1c} levels against the timeline of the DCCT and EDIC studies for the case and control subcohorts of Miao et al. (17). Circles (control group) and squares (case group) represent the approximate HbA_{1c} levels at the start of DCCT (1983), at year 10 of EDIC (2003), and at the time study participants donated blood for epigenetic analyses (2009/2010). Red and green bars represent the glycemic separation over the course of DCCT and EDIC. ChIP-chip was performed on lymphocytes (L) and monocytes (M) on three histone PTMs. Monocytes from case subjects (*right*) had a statistically significant higher number of histone H3 hyperacetylated regions as assessed by hybridization of ChIP on promoter tiling arrays. Proinflammatory genes, including *STAT1*, *IL1A*, and *TNFα*, were among the genes showing increased H3K9Ac in case subjects. H3K4Me3, H3 lysine-4 trimethylation; H3K9Me2, H3 lysine-9 dimethylation.

the setting of conventional versus intensive glycemic control, as in DCCT/EDIC, has not been investigated.

In this issue, Miao et al. (17) (including the DCCT/EDIC Research Group) profiled epigenetic changes in lymphocytes and monocytes obtained from a subcohort of DCCT/EDIC. The study design is robust with 30 case and 30 control subjects selected from the DCCT conventional and intensive groups. In a blinded fashion, Miao et al. analyzed histone activating (H3 lysine-9 acetylation [H3K9Ac] and H3 lysine-4 trimethylation) and repressive (H3K9 dimethylation) marks by chromatin immunoprecipitation (ChIP), followed by promoter tiling arrays analysis (ChIP-chip) covering a –3.2 to 0.8 kbp region relative to the transcriptional start site of all annotated human genes. A significant enrichment in H3K9Ac loci was observed in monocytes from case subjects—those who had been allocated to conventional therapy during DCCT. Importantly, monocyte H3K9Ac was associated with participants' glycemic history. The most hyperacetylated genomic regions in the case group included the proinflammatory genes *STAT1, TNFa,* and *IL1A*, as observed by ChIP-chip hybridization and validated by quantitative PCR analysis.

The novelty of Miao et al. is that epigenetic profiling of cells from DCCT/EDIC participants provides the first clinically relevant description of histone PTMs in T1D. This allows associations to be drawn between epigenetic changes and HbA_{1c} profiles over a 30-year period (Fig. 1). It should be noted, however, that by design, the DCCT/EDIC subcohort analyzed in this report has a marked HbA_{1c} separation between case and control subjects. It is therefore difficult to assess contributions to the epigenetic changes relative to more recent, milder, HbA_{1c} differences. Nonetheless, the authors show that conventional glycemic control induces long-lasting epigenetic modifications. Whether the observed epigenetic changes are causally associated with diabetes complications will require further scrutiny for two reasons. First, transcriptional and epigenetic changes were concordant in THP-1 monocytes exposed to high-glucose concentrations (18). The current study design, however, did not foresee systematic transcriptome profiling. Therefore, the functional relevance of the acetylation changes observed will need to be validated by performing gene expression studies. Second, because the interplay between DNA and its chromatic environment that regulates transcriptions is not limited to promoters, epigenetic changes linked to glycemic memory may also occur elsewhere in the genome. Even with this work ahead, the tremendous pace at which technologies in genomics advance, with ChIP-sequencing approaches that are becoming more affordable (19), will undoubtedly permit examination of the entire genome for hyperglycemia-induced epigenetic alterations in the near future. Miao et al. (17) demonstrated that an epigenetically based memory effect exists in monocytes from patients with T1D who withstood a period of poor glycemic control. Thus, as the DCCT/EDIC enters its fourth decade, we have initial evidence that the long-lasting protection against diabetes vascular complications that was gained by allocation to the intensive DCCT group can be understood, at least in part, in terms of epigenetic modifications.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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